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major portion of the dose (77-92%) of DBDPO. HPLC analysis of the skin homogenate extract detected primarily DBDPO and a smaller percentage of an unknown peak. TDCP was readily detected in the receptor fluid; 39-57% of the dose of TDCP was in the receptor fluid by 24 hr. The skin wash removed 11-25% of the dose of TDCP and 28-35% remained in the skin. HPLC analysis of the skin homogenate extract and receptor fluid extract from the TDCP high dose treated samples detected primarily TDCP and a smaller percentage of an unknown peak. Thus, TDCP more readily penetrates hairless mouse skin and diffuses into the receptor fluid than DBDPO. The differences in absorption between these chemicals may be due to their distinct physico-chemical properties (TDCP, log P=3.8, MW=431; DBDPO, log P=9.97, MW=960). These data do not provide information about the adverse effects of these chemicals, but they can be used to develop exposure or risk assessments. (This abstract does not reflect EPA or CPSC policy. Supported in part by CPSC-I-99-1167).

609 EVALUATION OF A QUANTITATIVE CLINICAL METHOD FOR ASSESSMENT OF SENSORY SKIN IRRITATION.

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Sensory skin irritation refers to the myriad of symptomatic complaints (*e.g.*, sting and burn) frequently associated with inflammatory skin conditions or skin intolerance to various products. Methods have been developed to quantitatively and objectively evaluate sensory irritation using a semantically-labeled log type scale of sensation magnitude (labeled magnitude scale or LMS). Using the LMS device, studies were conducted to determine if test subjects' perceptions of sensory response (from a series of survey questions) would predict their sensory reactivity to actual chemical challenge. The 15 survey questions included a broad range of sensation scenarios (*e.g.*, dipping hands into warm vs scalding water) that ranged from no sensation (LMS score=0) to the strongest imaginable sensation (LMS score=97). Individual variation and mean responses to each of the 15 questions were compared across studies. The mean LMS survey score was calculated for each subject. Considerable variation was seen between subjects' responses to the questions, particularly for questions pertaining to stronger stimuli (*e.g.*, scalding water). However, across six different study populations, the group mean scores for each question showed remarkable consistency. A positive correlation was also observed between the mean LMS survey scores and actual LMS responses to direct chemosensory chemical challenge for 80% of the subjects. Subjects with low mean survey scores required a higher threshold dose of the chemosensory irritant (*e.g.*, > 1000 uM Capsaicin) to elicit a moderate irritation score (LMS=16), whereas subjects with the highest mean survey scores reported moderate sensory responses at lower doses of Capsaicin (£ 1000 uM). Therefore, the mean LMS survey scores are consistent across study populations and show promise for identification of sensitive test subjects for chemosensory irritation testing.

610 INTEGRITY ASSESSMENT OF RAT AND HUMAN EPIDERMAL MEMBRANES FOR *IN VITRO* DERMAL REGULATORY TESTING: CORRELATION OF TRITIATED WATER PERMEABILITY WITH ELECTRICAL RESISTANCE.

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The *in vitro* dermal test guidelines provided by OECD recommend pre-study evaluation of membranes to confirm integrity by use of a marker molecule such as tritiated water (THO), measurement of electrical resistance (ER) or trans-epidermal water loss (TEWL). However, OECD's guidance document does not give quantitative criteria, but provides only a small collection of references and these are limited to a single threshold value for judging acceptability. A single threshold value lacks conservatism as only the most robust membranes are pre-selected. Rat and human epidermal membranes were prepared and mounted onto water-jacketed static diffusion cells (0.64 cm2) maintained at 32°C and a THO permeability coefficient (Kp) determined at 2, 4 and 6 hours. In separate experiments, ER was determined prior to and following a 2-hour THO Kp measurement. Following application of an infinite dose of THO, steady-state penetration was achieved by two hours. The distribution of THO Kp values for rat membranes exhibited a log-normal distribution; the model describing rat membranes set bounds of 0.75 to 10.2 x 10-3 cm/h enclosing 90% (Q5-Q95) of the data with a median value of 2.76 x 10-3 cm/h. The distribution of THO Kp data for human skin was described by a Weibull model. The model for human skin set bounds of 0.23 to 2.73 x 10-3 cm/h enclosing 90% of the measurements with a median THO Kp value of 1.13 x 10-3 cm/h. Two-hour THO Kp data were correlated with ER and the associated ranges for rat (4.2-12.9 k-ohms) and human (7.3-62.2 k-ohms) membranes estimated by inverse regression modeling with 95% fiducial bounds. The distribution of THO Kp data for rat and human epidermal membranes has been described and these data correlated with ER. The results provide reasonable and conservative measures for judging rat and human epidermal membrane integrity for *in vitro* dermal regulatory testing.

611 PERCUTANEOUS PBPK MODEL FOR THE UPTAKE OF PERCHLOROETHYLENE (PCE) FROM SOIL EXPOSURES IN RATS AND HUMANS.

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Extrapolation of percutaneous absorption from experimental animals to humans can be problematic. The goal of this research was to compare the dermal absorption of PCE from soil exposures in rats and humans using exhaled breath technology and PBPK modeling. Rat exposures were used to assess the effects of concentration, occlusion, and loading volume. Initially, a single homogenous dermal compartment was used to describe absorption. The skin permeability coefficient (K_p) for PCE in rats was 0.10 ± 0.02 cm/hr and did not vary between exposure scenarios. Non-occluded soil PCE concentrations declined to < 30% of original by 1 h, mostly due to volatilization. Human volunteers were exposed to PCE by submersing a hand in PCE-laden soil. Human exhaled breath profiles exhibited a slower absorption and elimination of PCE than the rat. The single dermal compartment was not sufficient to describe the slower human kinetics, thus two additional PBPK models with dual dermal compartments were developed: a parallel model to simulate follicular uptake, and a layered model to portray a stratum corneum barrier. The layered model provided the best fit to the human data. The human K_p value estimated for the dermal absorption of PCE was 0.0009 ± 0.0003 cm/h, over 100-fold lower than in rats, while the permeability coefficient for the transfer of PCE from the stratum corneum to the viable cutaneous tissue in the layered model (0.08 ± 0.01 cm/h) was very similar to the optimized K_p for rats. These data indicate that the stratum corneum layer of the hand may represent a barrier that results in decreased absorption in humans. Since rats often exhibit increased percutaneous absorption over humans, developing a model with the subcutaneous skin isolated from the viable cutaneous tissue may allow for improved extrapolation of rat absorption estimations for human risk assessment. (Supported by US DOE grant No. DE-FG07-97ER62509).

612 INFLUENCE OF DEET AND PYRIDOSTIGMINE BROMIDE ON DERMAL DISPOSITION OF PERMETHRIN.

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It has been suggested that the cause of the Gulf War Syndrome may be related to soldiers being exposed to insecticides (*e.g.*, permethrin, (P)), insect repellents (*e.g.*, DEET), and/or prophylactic treatment (*e.g.*, pyridostigmine bromide (PB)) against potential nerve gas attacks. However, it is not clear whether DEET and/or PB can influence the dermal bioavailabilty of co-administered insecticides. The purpose of this study was to assess the dermal disposition of permethrin in the isolated perfused porcine skin flap (IPPSF) model with simultaneous dermal exposure to DEET and systemic exposure to PB. IPPSFs were exposed to the following ethanol (E) and ethanol-water (E+W) mixtures (1.5:1) topically with and without PB in the perfusion media: P+E, P+E+DEET, P+E+W, P+E+W+DEET. The results indicated that PB significantly increased permethrin absorption (0.04 -0.11% dose *vs.* 0.22 - 0.26% dose), but had no effect on DEET absorption (0.08 - 0.13% dose). DEET significantly increased permethrin absorption in P+E mixtures only (0.27% dose *vs.* 0.17% dose), but had no effect on permethrin absorption with other mixtures, and its presence did not appear to influence the enhancing effect of PB on permethrin absorption. PB significantly increased permethrin in the stratum corneum (SC) in aqueous mixtures only (4.35 *vs.* 9.4% dose), while DEET appears to significantly reduce permethrin levels in the SC (1.35 - 2.54% dose *vs.* 9.4 -10.27% dose). PB also significantly enhanced permethrin penetration into all skin tissues and perfusate with ethanol-water mixtures in the absence of DEET. These experiments suggest that PB interactions in the dermal vasculature and DEET interactions on the

skin surface may alter the disposition of permethrin in skin and possibly its bioavailability in soldiers simultaneously exposed to these chemicals if these results mimic human exposure. (Supported by USAMRMC Grant, DAMD-17-99C-9047).

613 STEREOSELECTIVE ABSORPTION OF PERMETHRIN THROUGH SILASTIC MEMBRANE AND EXCISED PORCINE SKIN *IN VITRO* FLOW THROUGH DIFFUSION SYSTEM.

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Permethrin has four isomers, two of which have insecticidal activity (1R-cis- and 1R-trans-). During the Gulf War, soldiers were exposed to certain insecticides that included permethrin as well as other chemicals. The purpose of this study was to determine if differential absorption occurs between trans-permethrin and cis-permethrin in the silastic membrane and the excised porcine skin in an *in vitro* flow through diffusion system. The silastic membrane and porcine skin was dosed with 14C-permethrin containing approximately 48+/-1% of the trans-permethrin isomer and 52+/-1% of the cis-permethrin isomer. Perfused samples were collected at various time points over an eight hour period. Representative samples from the 90 minute, 8 hour and peak absorption times were extracted and the isomers were separated by HPLC. Based on total radioactivity, preliminary results suggest that the trans-permethrin isomer is absorbed more readily than the cis-permethrin isomer in the silastic membrane. Work is currently underway to investigate absorption in the excised porcine skin. (Supported by USAMRMC Grant, DAMD-17-99C-9047).

614 DERMAL UPTAKE OF PESTICIDES DURING EXPOSURE EVENTS WITH INTERMITTENT SURFACE CONTACT: MALATHION CASE STUDY.

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The goal of this study is to select a dermal uptake model that simulates dermal adsorption and absorption of pesticides such as malathion on the hands following short-term contact, and is compatible with PBPK models. Existing dermal uptake models address uptake of chemicals from relatively long-term (near steady-state conditions) during contact with water, soil, and other carrier vehicles (*e.g.*, creams, cloths). These models are not appropriate for assessing uptake from short-term (seconds to minutes) and intermittent contacts with chemical residues on surfaces treated with liquid, powder and granular pesticide. To address this issue, we developed a model to predict diffusive fluxes of compounds through the epidermis using a finite-difference discretization applied to Fick's second law. The model can estimate the skin uptake as a function of time and depth and is calibrated with studies on the rate of malathion penetration in rats. Tape stripping data were collected after 1, 4, and 12 hours of exposure. Both the newly developed discretized model and equivalent one-compartment models are evaluated for predicting the observed exposure and uptake. The fully discretized model was used to develop an equivalent, but simpler compartment model that can be linked with PBPK models. This model estimates total uptake to blood from transient contacts using steady-state permeability (K_p), skin loading, a partition factor and contact time. Although total uptake can be a nonlinear function of contact duration, we have discovered a range of contact times and contact regimens for which uptake to blood is not sensitive to boundary assumptions and is a simple linear function of cumulative contact. We are developing both in vivo and *in vitro* protocols to continue model evaluation. This work has been funded wholly or in part by the United States Environmental Protection Agency. It has been subjected to Agency review and approved for publication.

615 EXPOSURE TO SULFUR MUSTARD CAUSES A CONCENTRATION DEPENDENT LOSS OF INHIBITORY ACTIVITY OF ALPHA$_1$ ANTITRYPSIN.

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Sulfur mustard (SM) is a potent vesicating agent that causes, in human skin, a separation at the dermal-epidermal junction leading to the formation of large fluid filled blisters. The mechanisms of blister formation have not been elucidated, but there appears to be proteolytic cleavage at the sites of SM exposure resulting in large fluid filled bullae. Possible mechanisms for the proteolytic cleavage at these sites of SM exposure are the decrease in protease inhibitor capacity and/or the increase in protease activity. To determine whether SM exposure could initiate loss of protease inhibitor activity, alpha$_1$ antitrypsin (α_1-AT), the major antiprotease at the dermal-epidermal junction, was exposed to various concentrations of SM and its resulting inhibitory capacity was determined. The effect of SM on α_1-AT inhibitory capacity was measured by incubating α_1-AT with various concentrations (10^{-4} to $2x10^{-3}$ M) of either SM or hydrolyzed SM for 1 hr at 37° C. The resulting α_1-AT solutions were mixed with trypsin for 30 mins at 37° C before being added to a 96-well plate containing benzoyl-DL-arginine-p-nitroanilide (BAPNA), a trypsin substrate, and the optical density of the wells was determined at 405 nm. The loss of the ability of SM-treated α_1-AT to inhibit trypsin was determined by the increase in optical density from the cleavage of benzoyl-DL-arginine-p-nitroanilide by the still active trypsin. Loss of α_1-AT activity was detected at concentrations as low as $3x10^{-4}$M SM and reached its maximum of a 30% decrease at $1.5x10^{-3}$M SM. The hydrolyzed SM did not demonstrate any ability to decrease the inhibitory activity of α_1-AT. It appears that SM can cause a decrease in the inhibitory activity of α_1-AT, but it has not yet been determined whether this SM initiated decrease in inhibitory capacity is significant enough to result in increased proteolytic activity at the dermal-epidermal junction and the formation of blisters.

616 TOXICOKINETICS OF TOPICALLY APPLIED SULFUR MUSTARD IN THE FUR-COVERED AND HAIRLESS GUINEA PIG SKIN: EFFECT OF IODINE AND HYPOCHLORITE.

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Sulfur mustard (SM) is a powerful vesicant used as chemical warfare. In the present study skin levels of SM were quantitatively measured in the fur-covered and hairless guinea pig models. Wells were constructed on the back of an anesthetized animal by the following procedure. A plastic tube was cut to form open-ended cylindrical wells and a thin layer of commercial silicon sealing ointment was applied to one edge of the wells. The wells were then attached to the animals back so that liquid inside the wells did not leak out. The center of each well was exposed to 1.2mg SM (neat liquid). At certain time intervals after exposure methylene chloride (0.5 ml) was applied into each well for extraction of SM from the skin of the living animal. SM was quantified by gas chromatography/mass spectrometry analysis. Measurements taken 30 and 60 min after exposure of male guinea pig showed SM reduction by 44% and 99.4%, respectively. In the female the rate of SM disappearance was slower, namely, decrease of 39%, 82% and 99.6% was observed 60, 120 and 180 min after exposure, respectively. Iodine and sodium hypochlorite did not significantly alter skin SM levels in comparison to the effect of their vehicles. The male hairless guinea pigs showed similar pattern to that observed in the haired guinea pigs. Low levels of skin SM were detected after termination of exposure to SM vapor in both animal models. These findings indicate that the rate of penetration of neat liquid SM and its reaction with skin components is within tens of minutes and even hours after exposure. The fact that iodine does not affect the vesicant is important for understanding the mechanism of iodine-induced protection against SM. (supported by the U.S. Army Medical Research and Material Command under Cooperative Agreement No. DAMD17-98-2-8009).

617 GENE ARRAY ANALYSES OF SULFUR MUSTARD-INDUCED INFLAMMATORY MEDIATOR RESPONSE IN MOUSE EARS.

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As part of the Medical Chemical and Biological Defense Research Program, the U.S. Army Medical Research Institute of Chemical Defense has a mission to identify biomarkers predictive of exposure to vesicating agents such as sulfur mustard [bis(2-chloroethyl) sulfide; HD]. Previous reports from this laboratory established that HD influences inflammatory gene expression in several *in vivo* models of vesicant injury. Gene expression arrays were used to profile HD-induced gene expression in ear tissue of CD1 mice. Adult, male mice were treated topically with HD (0.16 mg) on the medial surface of the ear. At 3, 6, 12, or 24 hr following exposure, biopsies were collected. RNA was isolated and complementary deoxyribonucleic (cDNA) probes were radioactively labeled using gene-specific primers. Labeled cDNA probes were hybridized to 588-gene expression arrays and phosphorimager